THE PERFORMANCE AND CAPABILITIES OF TERRESTRIAL ORGANISMS IN EXTREME AND UNUSUAL GASEOUS AND LIQUID ENVIRONMENTS

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Prepared under
Grant No. NGL 12-001-042
with the
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

SEMI-ANNUAL REPORT

Growth and Development in Inert Non-aqueous Liquids

January 1974

University of Hawaii
Hawaii Botanical Science Paper
No. 36

Informal technical report, neither to be cited nor credited as a publication.

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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Grant No. NGL 12-001-042

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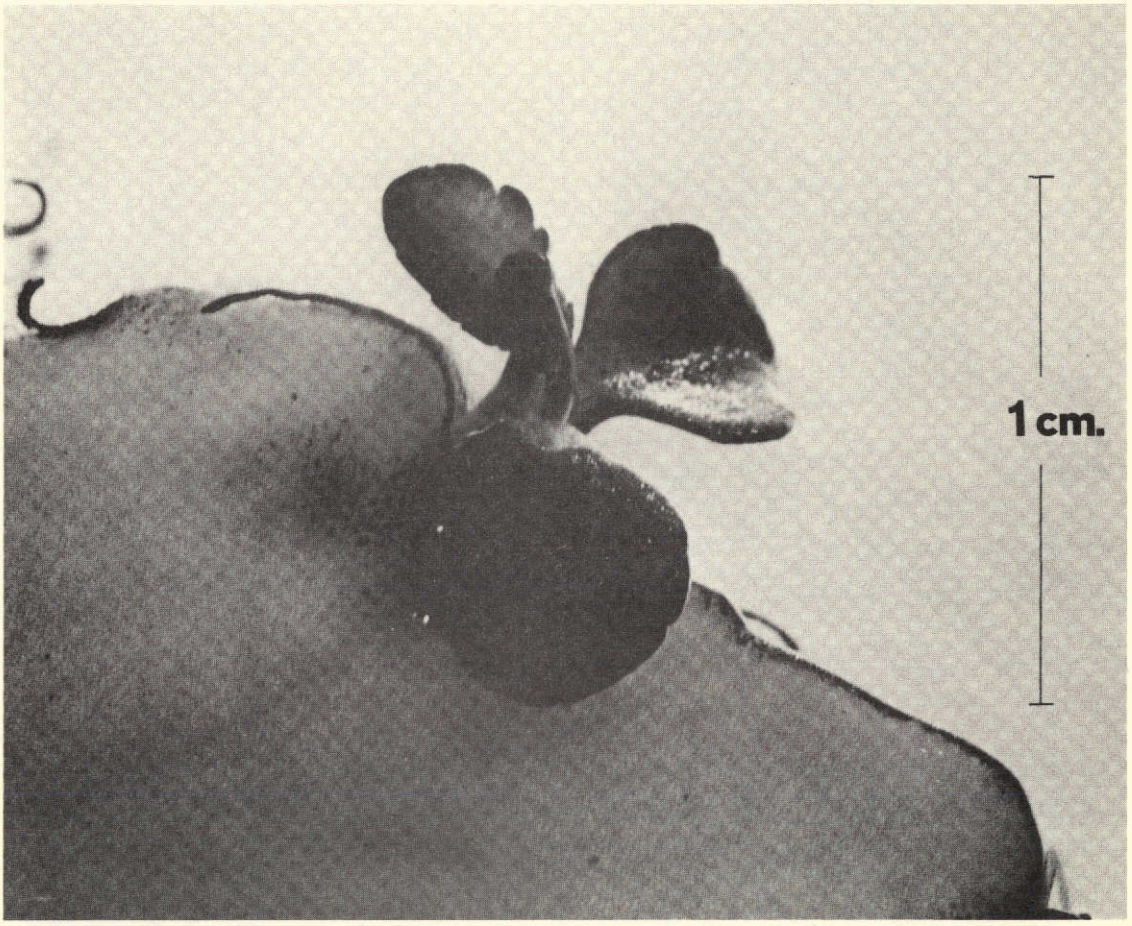

Frontispiece. Plantlet of *Bryophyllum diagremontianum* which developed over the course of 20 days while completely submerged under mineral oil. It is attached to a leaf.

Introduction

This report contains a preview of the survival and growth capabilities of higher plants in non-aqueous, inert liquids. The two media which were used are mineral (white) oil and fluorochemical inert liquid FC-75. Both liquids dissolve oxygen and carbon dioxide readily, but are insoluble in water. Consequently, plants submerged in these liquids are capable of gas exchange with the atmosphere, but possess a water impermeable coating the dimensions of which are determined by the size of the liquid holding container. In a sense, growing plants in a tank of mineral oil imparts on them a "cuticle" many centimeters thick.

Studies of free water deficit plant relations can be greatly facilitated using inert fluids. The ability of many higher plants to survive and grow in submarine environments was reported in Hawaii Botanical Science Paper No. 5. In this present study, plants plus prescribed volumes of water were innoculated into mineral oil. Organisms with minimal water supplied could then be observed. Also, submersed plants covered with an oil slick were shown to be capable of growth in dessicating atmospheres.

GERMINATION IN MINERAL OIL

To provide an adequate survey of germination responses for higher plants, members of three families which are divergent in most toxonomic schemes were chosen. These families are the Cruciferae, Gramineae and Labiatae. In the Cruciferae the following *Brassica* (cauliflower, rutabaga, turnip, mustard and kohl rabi), *Raphanus* (radish), *Iberis* (candytuft), and *Alyssum* (alyssum) were used. Gramineae included *Zea* (corn), *Secale* (rye) and *Hordeum* (barley). *Salvia* of the Labiatae was also used.

A preliminary investigation of the hydration and germination response of these seeds on wet filter paper was conducted. When a dry seed is placed in a moist environment it usually absorbs water in three stages:

1) an initial period of rapid uptake, which is the result of physical absorption of water by colloidal materials in seeds, occurring in dead as well as living seeds;
2) a lag period in which little water is absorbed; and
3) a second uptake stage which is associated with embryo growth.

Each family has a distinctively different hydration response. All Cruciferae (Figures 1-8) show a prolonged lag phase. The lag for the Gramineae is extremely short (Figures 9, 10, 11). *Salvia* (Figure 12) shows a decrease in fresh weight before the onset of

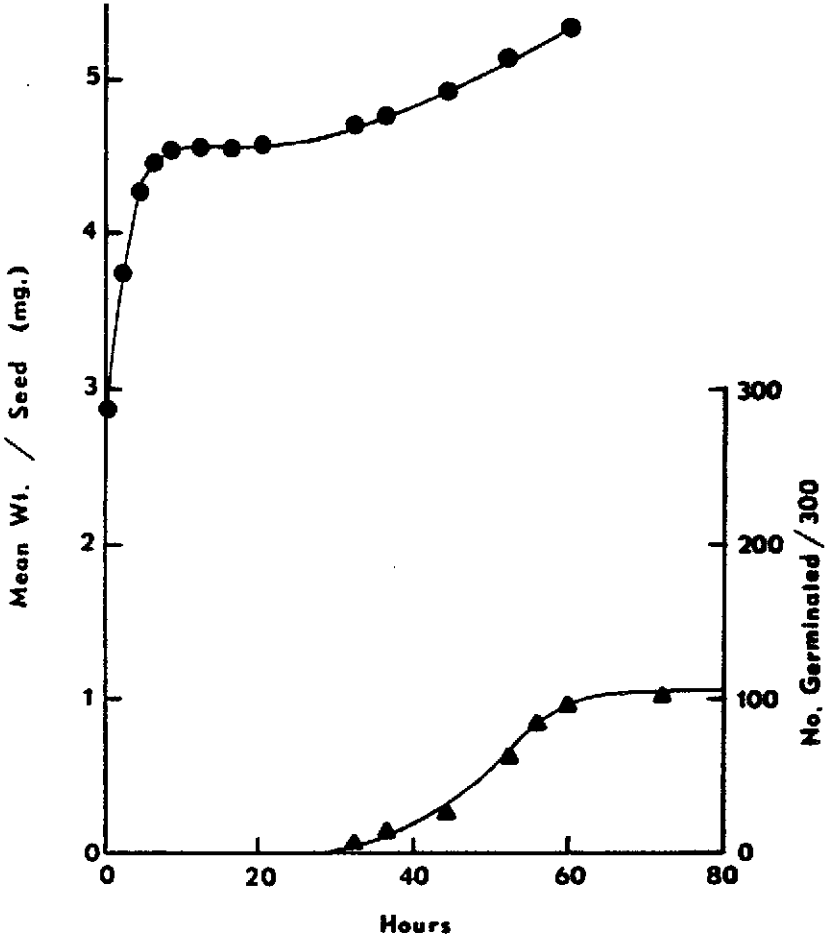

Figure 1. Cauliflower germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

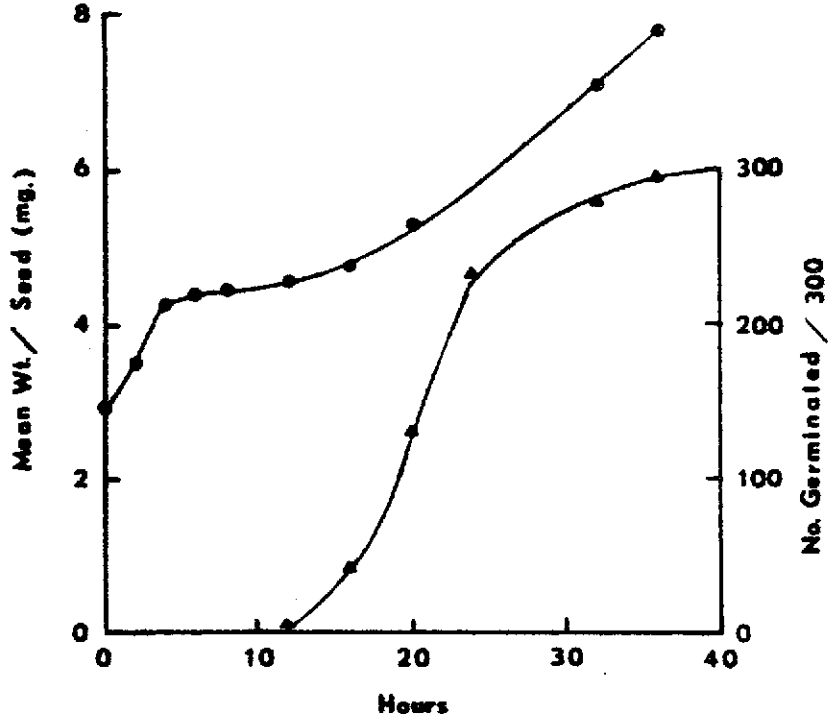

Figure 2. Rutabaga germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

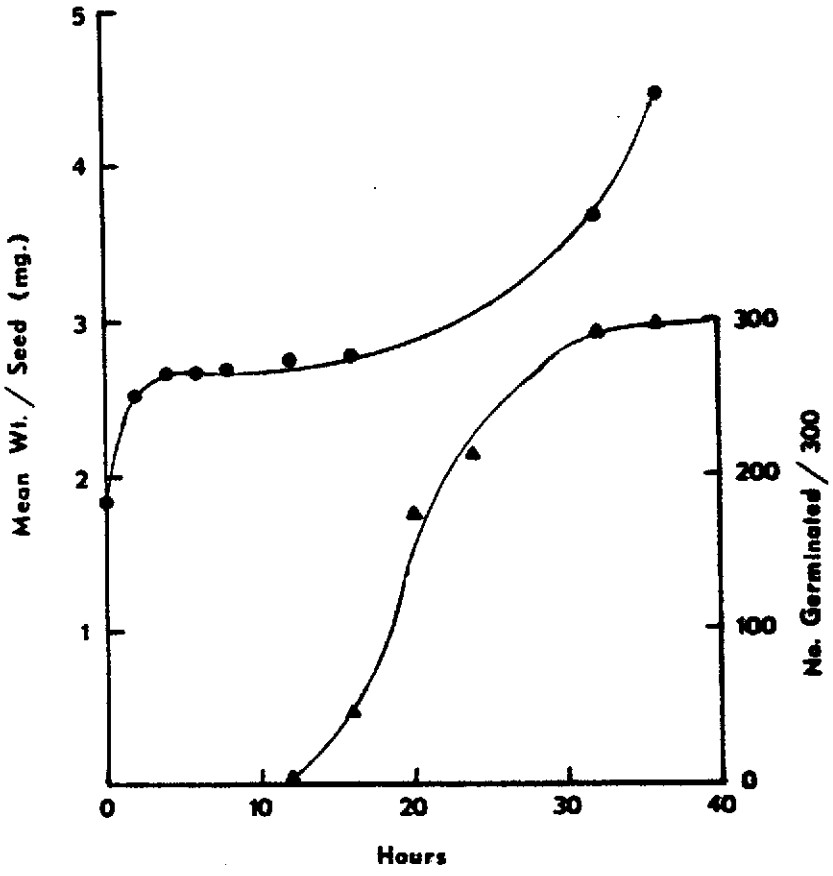

Figure 3. Turnip germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

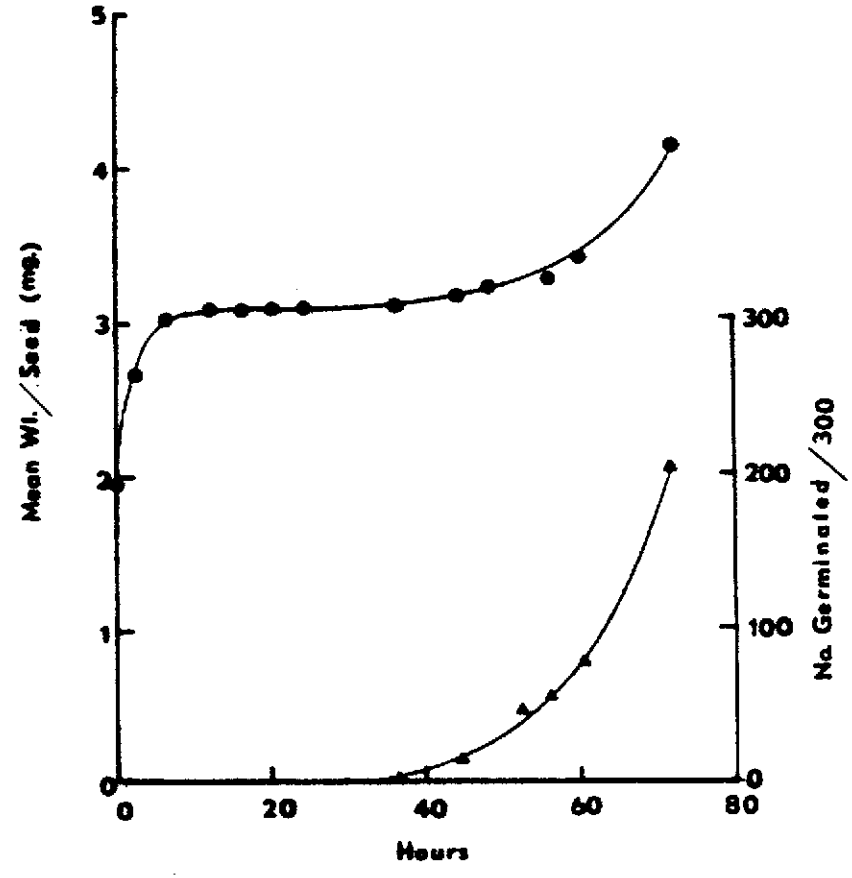

Figure 4. Mustard germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

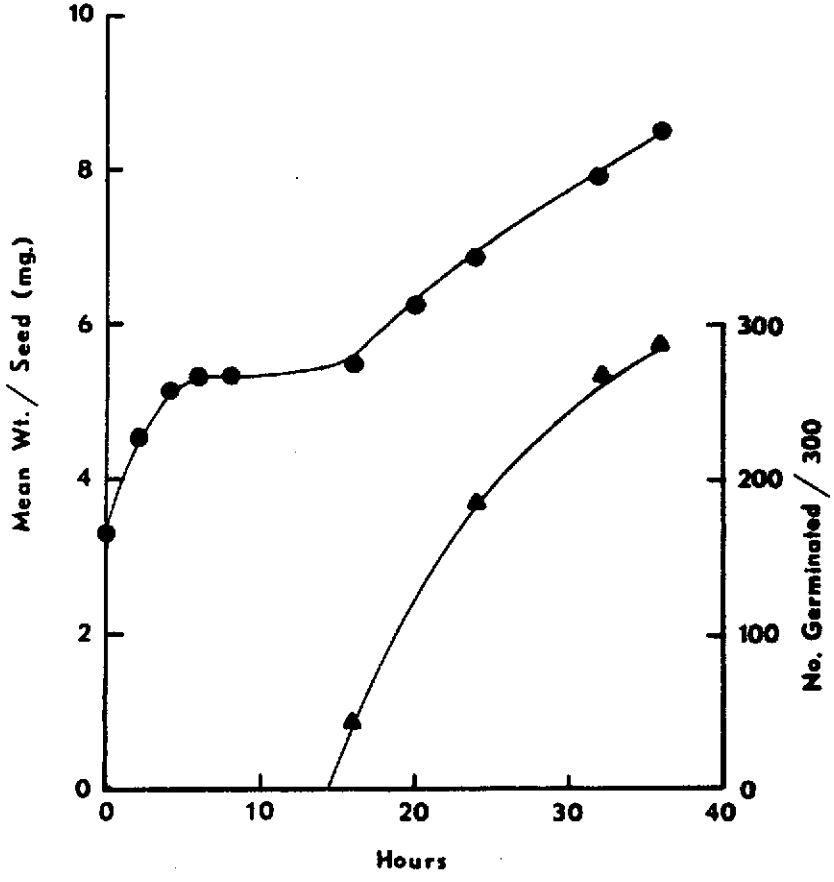

Figure 5. Kohl rabi germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

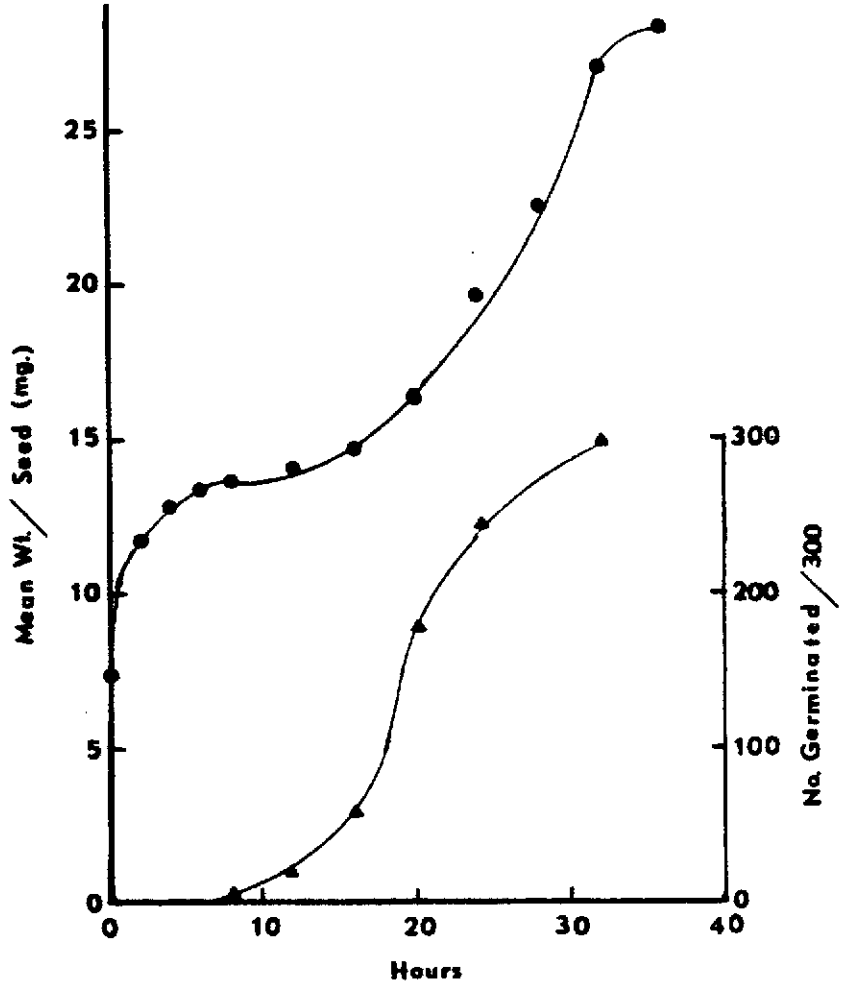

Figure 6. Radish germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

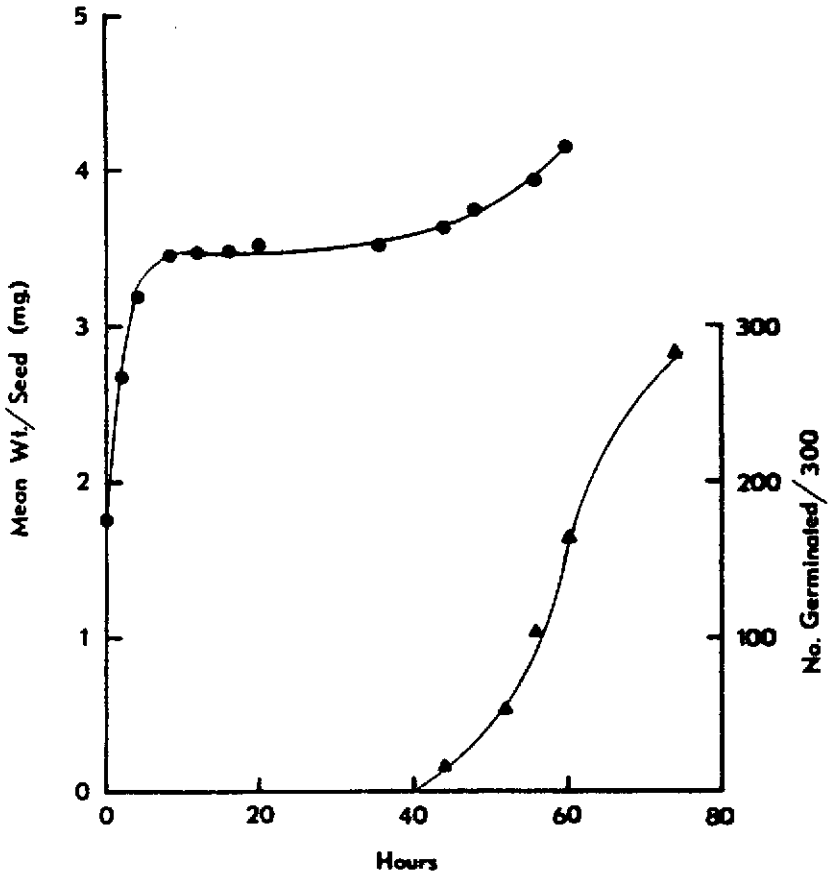

Figure 7. Candytuft germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

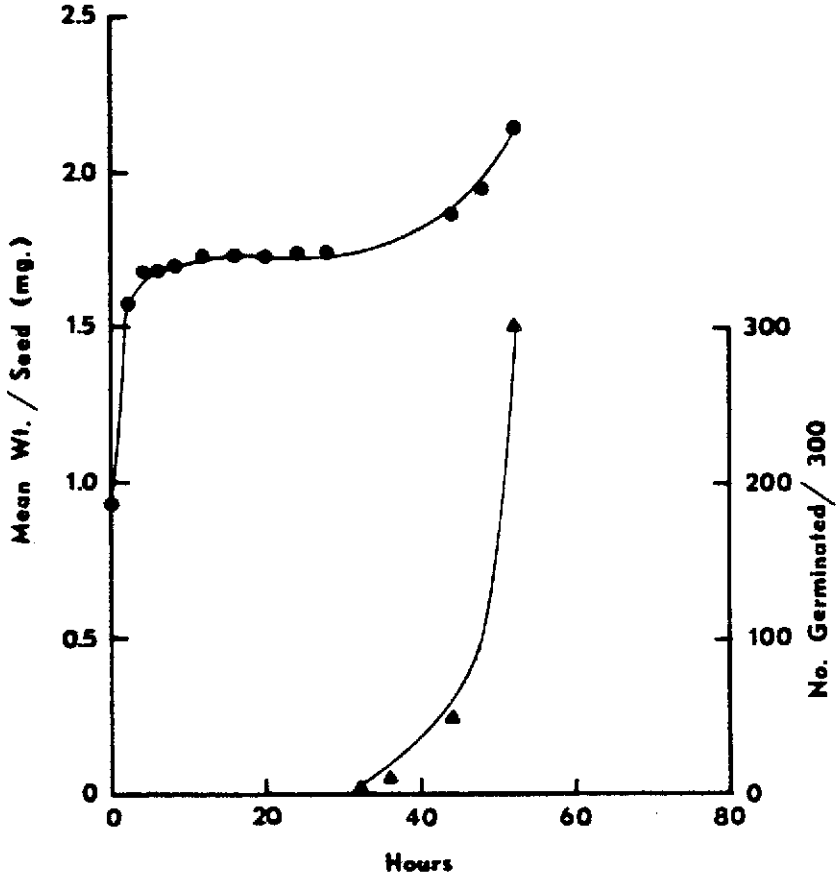

Figure 8. Alyssum germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

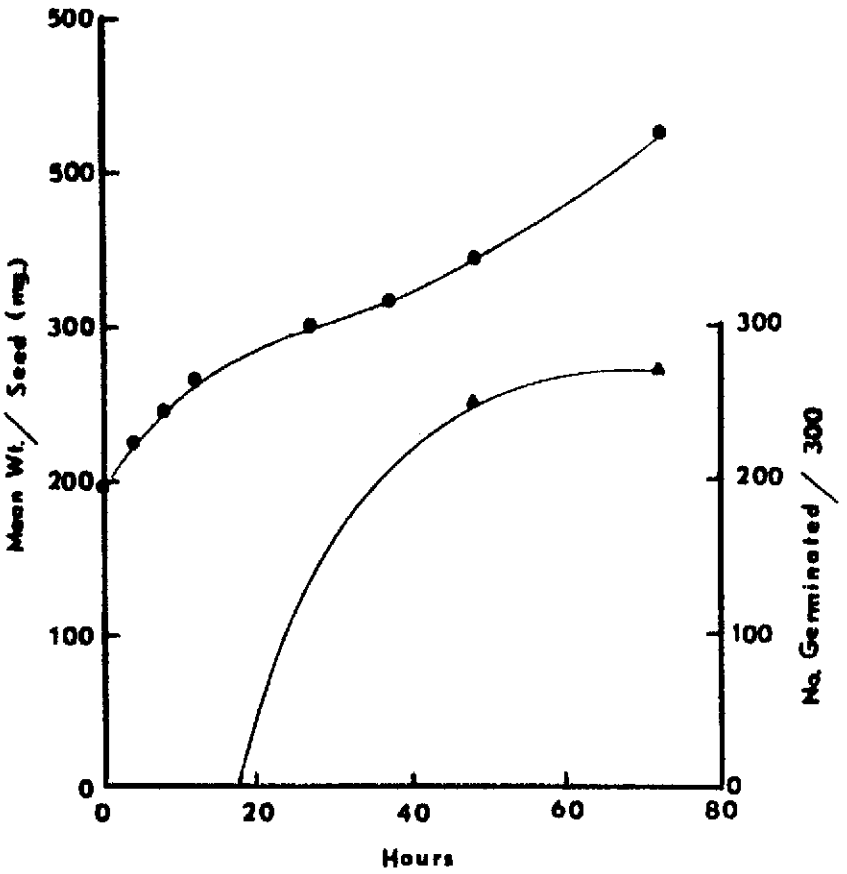

Figure 9. Corn germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

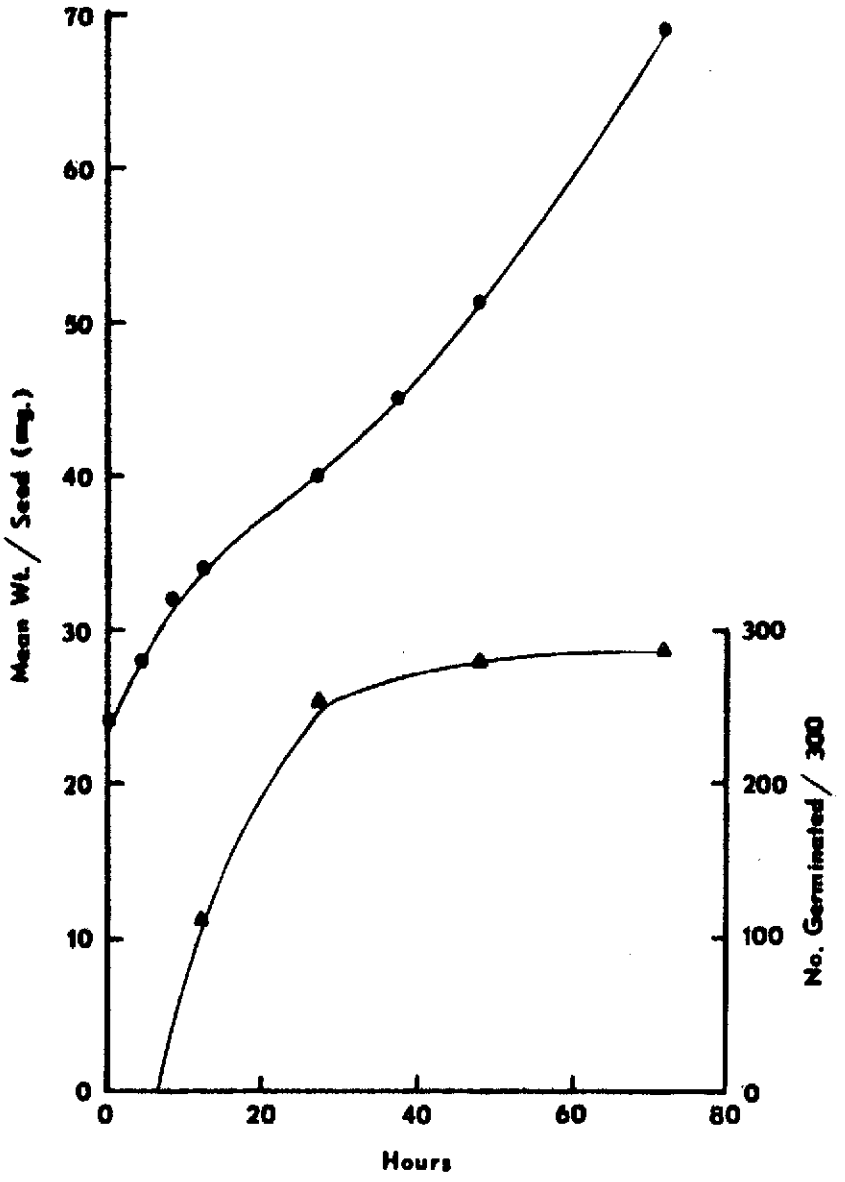

Figure 10. Rye germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

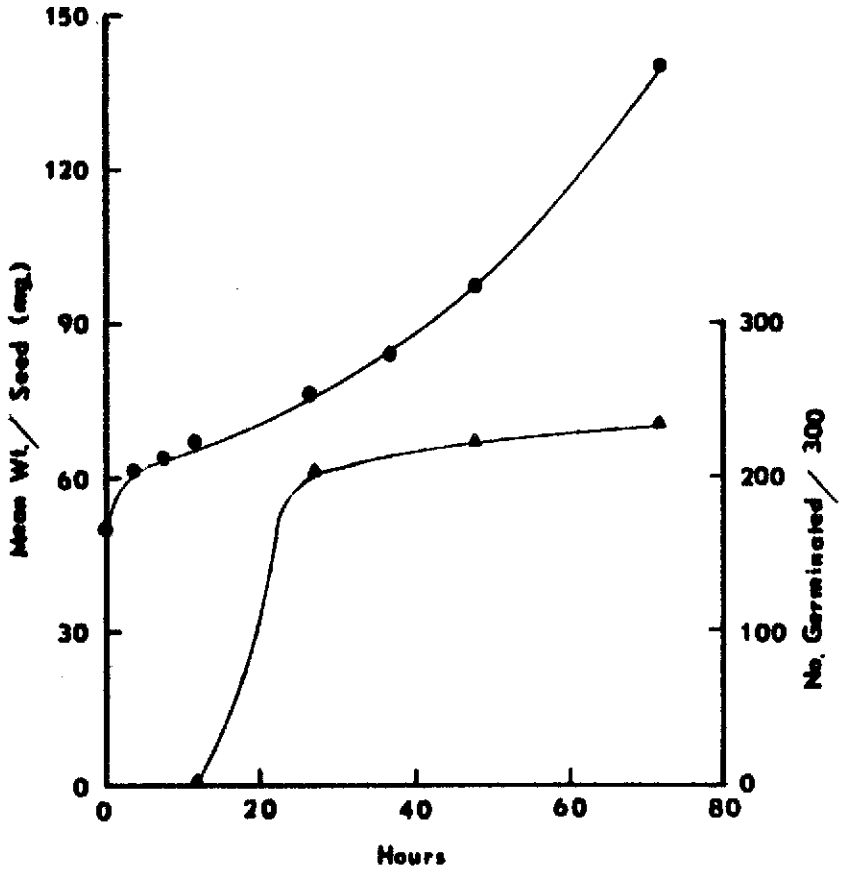

Figure 11. Barley germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

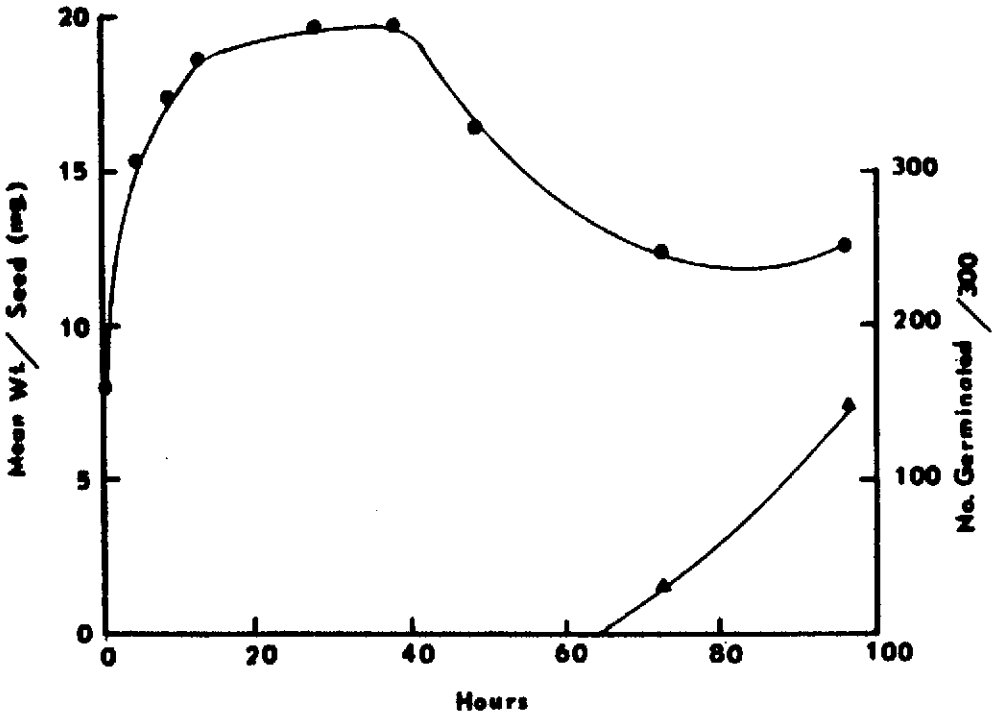

Figure 12. *Salvia* germination on moist filter paper under constant 100 foot candles of fluorescent light. The mean fresh weight per seed (circles) and the number germinated per 300 seeds (triangles) is plotted as a function of time.

germination. This is apparently due to the dissolution of a gelatinous sheath surrounding the seed coat.

To test germination responses in mineral oil, the following procedure was used. A period lasting half of the duration of the initial rapid water uptake phase was chosen to hydrate the seeds on moist filter paper. They were then blotted on dry filter paper, air dried for five minutes, and placed in petri dishes with a 5 mm covering of mineral oil. The extent of germination after 7 days is shown in Table 1. No growth occurred after this time.

The length to which some of the radicles emerged is notable (see Figures 13 and 14).

Considering all the species tested, there is an increasing germination trend which is related to the % increase in imbibed seed weight prior to mineral oil immersion. Within the Cruciferae, rutabaga, turnip and mustard showed no sign of germination. Germination of alyssum was 21 percent in mineral oil. For mustard, candytuft, cauliflower and radish, there is a linear correlation significant at the 5 percent level between percent germination in mineral oil and the duration of the absorption lag in H_2O (Figure 15); the shorter the lag, the greater the germination percentage. No significant relationship was found between germination in mineral oil and dry weight of seeds (Figure 16), nor with the period in water before germination occurs (Figure 17) for seeds on wet filter paper.

Table 1. Germination of Cruciferae, Gramineae and Labiatae after hydration for prescribed time and then immersion in mineral oil for seven days.

	Hydration (hrs.)	After 7 days Number/100 germinated
Cruciferae		
cauliflower	2	0
rutabaga	2.5	0
turnip	2	0
mustard	3	3
kohl rabi		0
radish	3	8
candytuft	4	4
alyssum	2	21
Gramineae		
corn	11	0
rye	4	0
barley	6	3
Labiatae		
Salvia	10	6

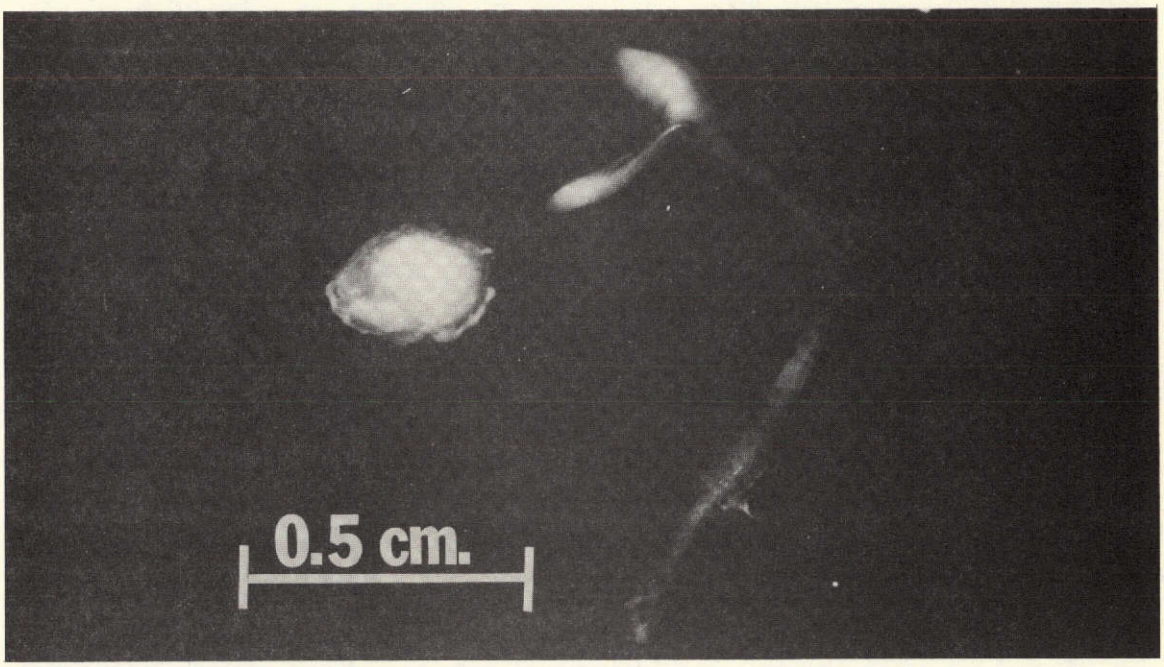

Figure 13. Alyssum plantlet under mineral oil. Seeds were hydrated for two hours, air dried for five minutes and then placed in mineral oil for seven days. Both germination and elongation occurred in mineral oil. Stem and cotyledons are dark green.

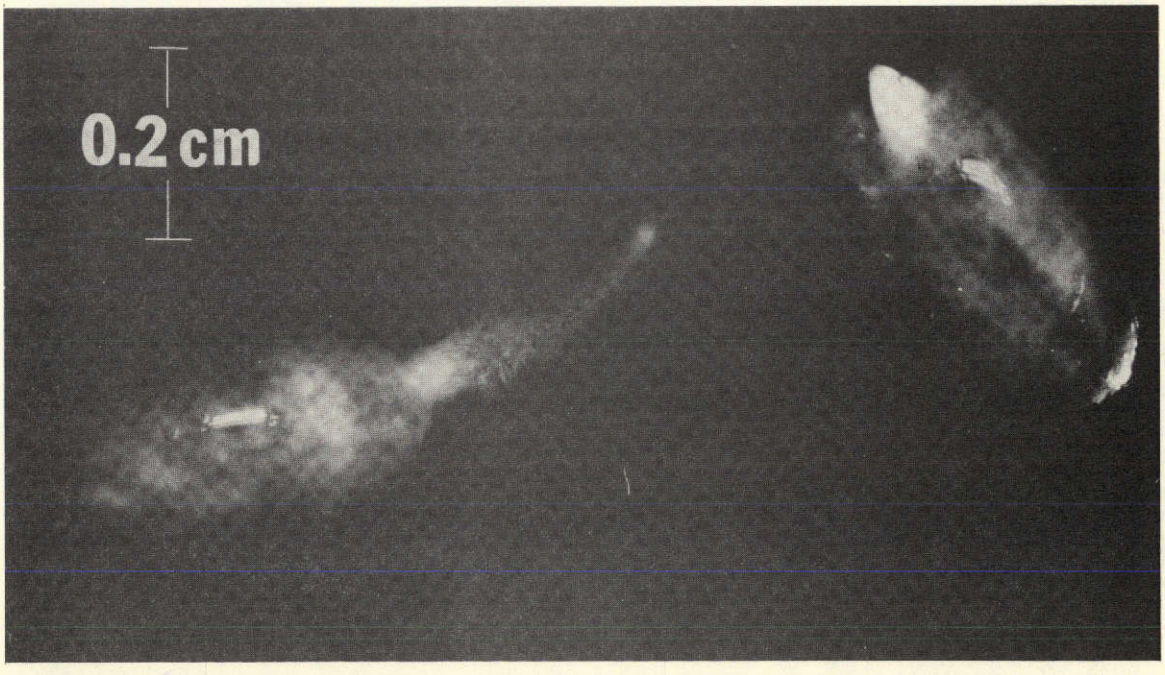

Figure 14. Germinated *Salvia* in mineral oil. Seeds were hydrated for ten hours, air dried for five minutes and then placed in mineral oil for seven days. Germination occurred in the mineral oil.

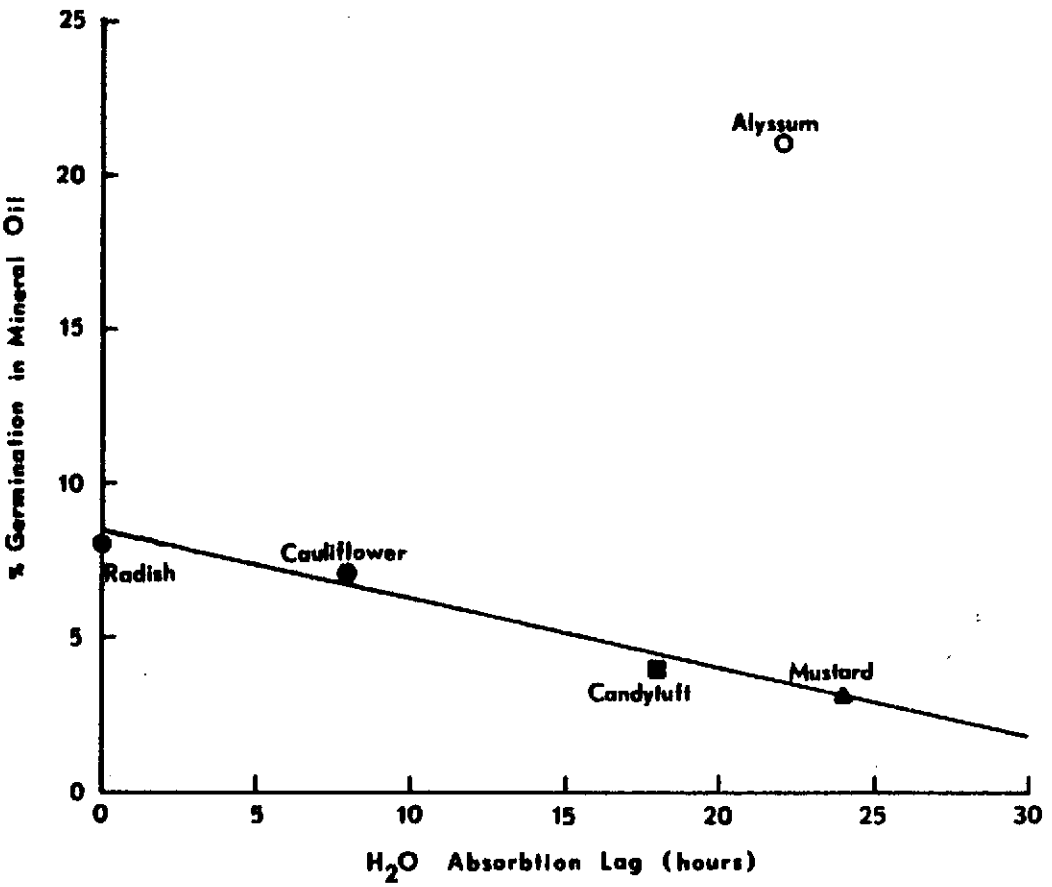

Figure 15. Percentage of Cruciferae which germinated in mineral oil is plotted against the duration of the water absorption lag on moist filter paper. For radish, cauliflower, candy tuft and mustard, the linear $r = 0.98$, $p < 0.05$.

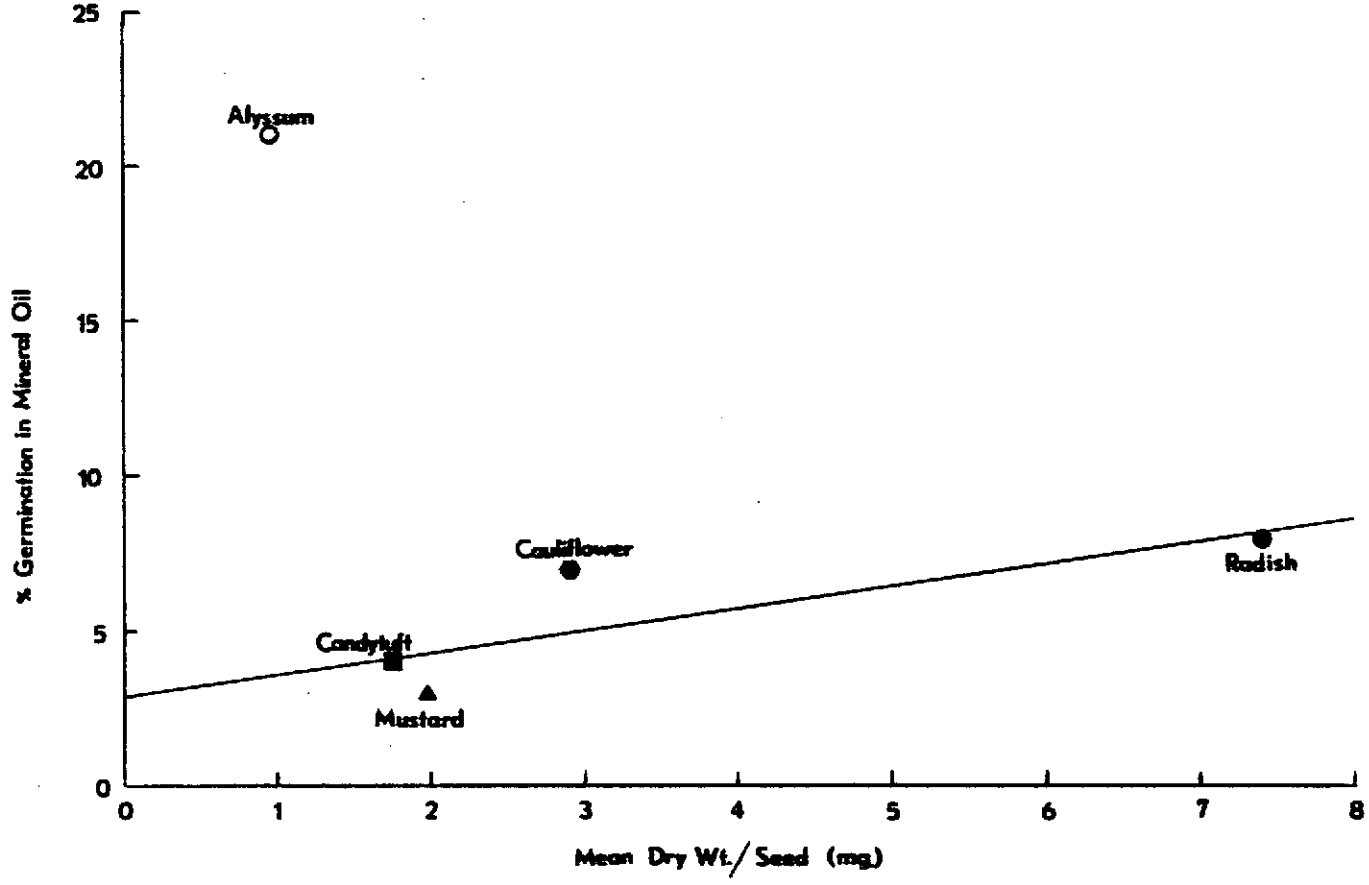

Figure 16. Percentage of Cruciferae which germinated in mineral oil is plotted as a function of the mean dry weight per seed. For radish, cauliflower, candy tuft and mustard, the linear $r = 0.81$, $p > 0.05$.

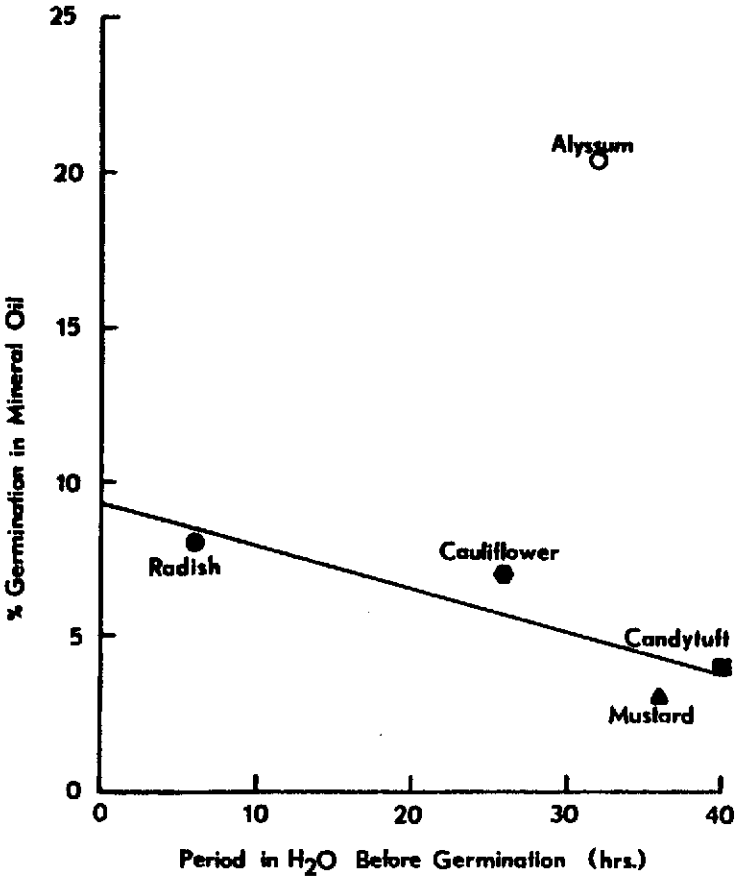

Figure 17. The percentage of Cruciferae which germinated in mineral oil is plotted as a function of the length of time on moist filter paper necessary for germination to occur. For radish, cauliflower, candy tuft and mustard, the linear $r = 0.88$, $p > 0.05$.

GROWTH OF SUBMERGED RADISHES UNDER ARID CONDITIONS

AND REDUCED ATMOSPHERIC PRESSURES

As a result of the low solubility of oxygen in water, these two compounds compete for physical space in the environment of a submerged seed. The response of radishes to aeration, as well as reduced partial pressures of CO_2, O_2 and H_2O were tested. This was done in three different liquid milieus:

a. water,

b. water + mineral oil,

c. water + mineral oil + fluorocarbon.

The specific gravity of mineral oil is 0.9, while that of liquid fluorocarbon is 1.5. One thousand ml-beakers were used, with 25 seeds per beaker. They contained 200 ml H_2O ± 500 ml mineral oil ± 100 ml fluorocarbon. The four treatments were:

a. 1 atmosphere + aeration,

b. 1 atmosphere, no aeration,

c. 1/2 atmosphere (vacuum),

d. N_2 atmosphere.

See Table 2 for results after 7 days.

The following observations can be made:

1) When subjected to a N_2 atmosphere or 1/2 atmosphere, plants will only germinate if covered with mineral oil.

Table 2. The response of radish seeds after seven days under different liquid and atmospheric conditions is shown. At all times, the bulk of the seed or plantlet was in the H_2O layer due to their similar specific gravities. Relative total chlorophyll content was computed after acetone extraction and spectrophotometric measurement using the equation total chlorophyll = $20.2\ OD_{645} + 8.02\ OD_{663}$.

Environment		# germinated per 25	Average fresh wt. (mg)	Mean length (mm)	Relative amount of chlorophyll
Atmosphere	Liquid				
1 atmosphere, (bubble)	H_2O	25	132	127.7±21.3	4.30
	H_2O + oil	24	92	78.4±30.8	2.15
	H_2O + oil + fluoro.	21	48	29.8±20.6	1.84
1 atmosphere, (no bubble)	H_2O	24	64	27.1±16.4	1
	H_2O + oil	24	56	14.0± 7.5	1.56
1/2 atmosphere	H_2O	DEHYDRATED			
	H_2O + oil	9	16	1.4± 2.5	0.41
N_2 atmosphere	H_2O	0	8	0	0.44
	H_2O + oil	6	44	1.5± 2.9	1.92

Mineral oil could be used for culturing plants submersed in water on a planet with reduced atmosphere such as Mars.

2) At one atmosphere with aeration, mineral oil impedes growth only slightly, without affecting germination. There is approximately a 50 percent chlorophyll increase with mineral oil.

3) One week radish seedling germinated in containers containing mineral oil, H_2O, and fluorocarbon layers (Figure 18) lie predominantly in water layer, with hypocotyls protruding into fluorocarbon.

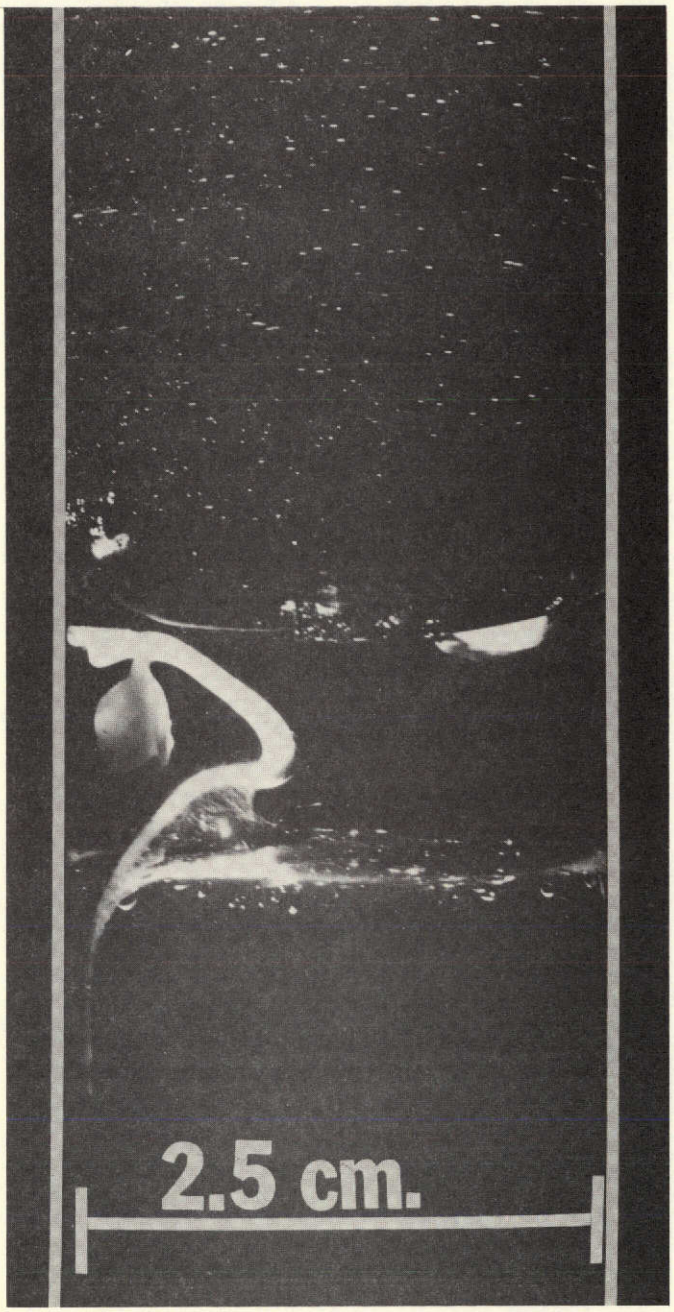

Figure 18. Submerged and aerated radish seedling sowed one week previous. Bottom layer is fluorocarbon liquid (sp. gr. 1.5), central layer is water, and upper layer is mineral oil (sp. gr. 0.9).

CRASSULACEAN AND SUCCULENT SURVIVAL IN MINERAL OIL

The capacity of succulent and crassulacean plants to take extreme H_2O stress in mineral oil was tested. *Bryophyllum calycinum* leaves with attached plantlets, but no roots and *Bryophyllum diagremontianum* leaves with no plantlets were submerged in 6 liters of aerated mineral oil. The fluorescent light intensity was 500 candle power.

Figure 19 shows the extent of root emergence from the *B. calycinum* plantlets after 1 week. The *B. diagremontianum* plantlets shown in Figure 20 emerged over a period of 2 weeks. Figure 21 illustrates the appearance of one of the above *B. diagremontianum* plantlets after two weeks, compared to a plantlet from a leaf kept on wet filter paper for the same time period.

Immersion of *Styphelia* sp. (Figure 22) and Night Blooming Cereus (Figure 23) in mineral oil for one month did not affect their outward appearance.

ACKNOWLEDGEMENTS

Many thanks to Stephanie Chun and Lisa Shigeyawa for their kind labors in this project.

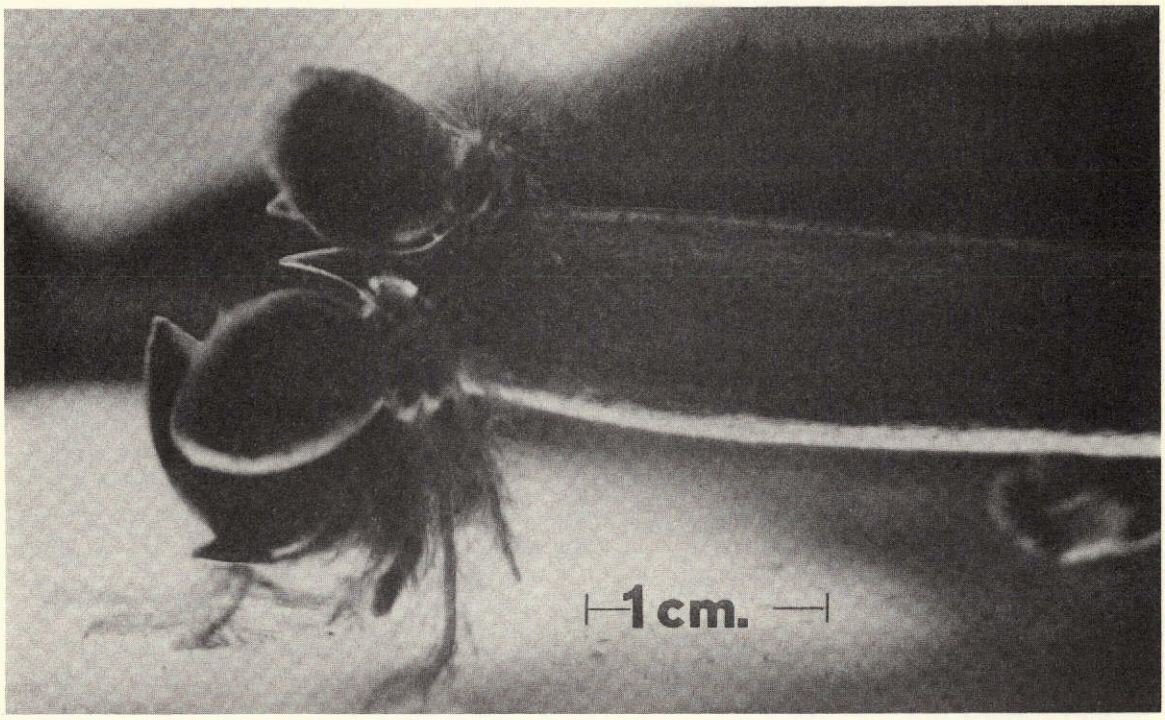

Figure 19. *Bryophyllum calycinum* leaf with plantlets submerged in mineral oil for one week. At the beginning of this time period, no roots were visible.

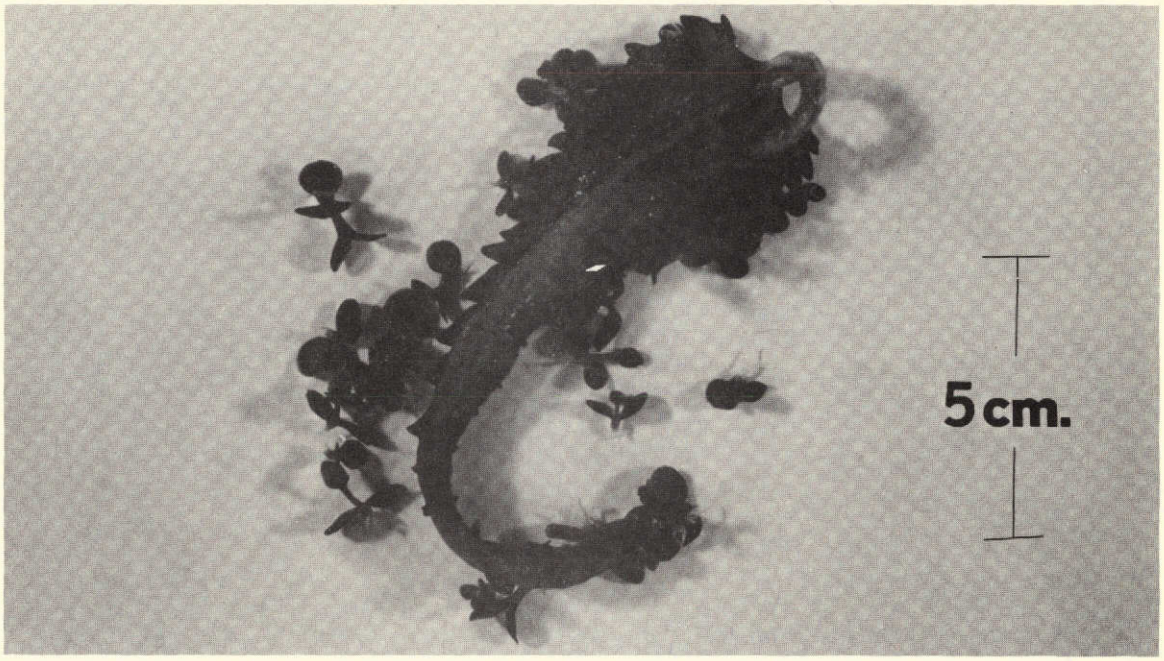

Figure 20. *Bryophyllum diagremontianum* leaf and plantlets after two weeks in mineral oil. No plantlets were present at the beginning of this period.

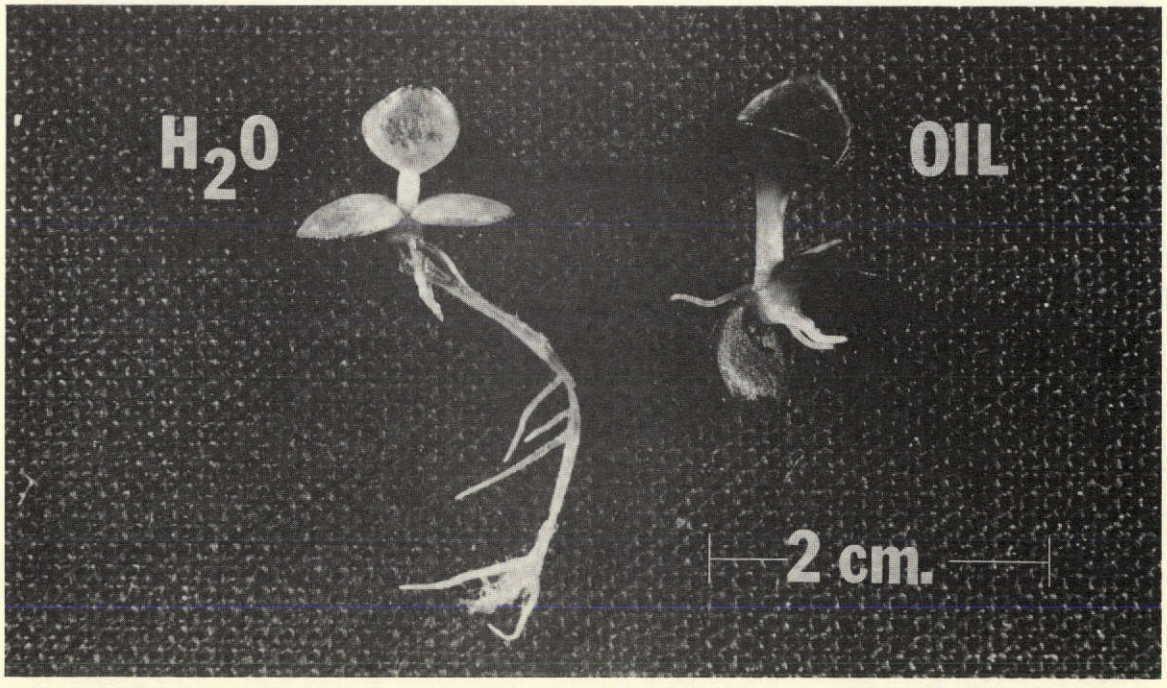

Figure 21. *Bryophyllum diagremontianum* plantlets. The plantlet on the left emerged over a period of two weeks from a leaf on moist filter paper. The plantlet on the right (shown also in Figure 20) emerged over the same period from a leaf submerged in aerated mineral oil.

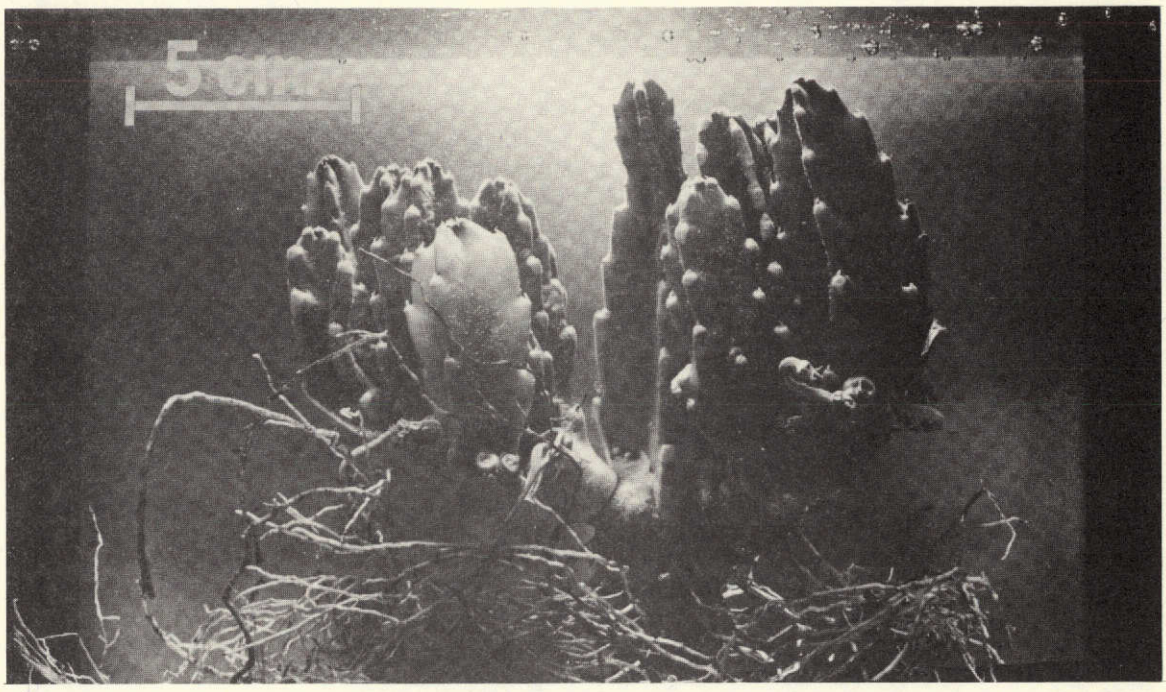

Figure 22. *Styphelia sp.* immersed in aerated mineral oil for one month, with no apparent change in outward appearance.

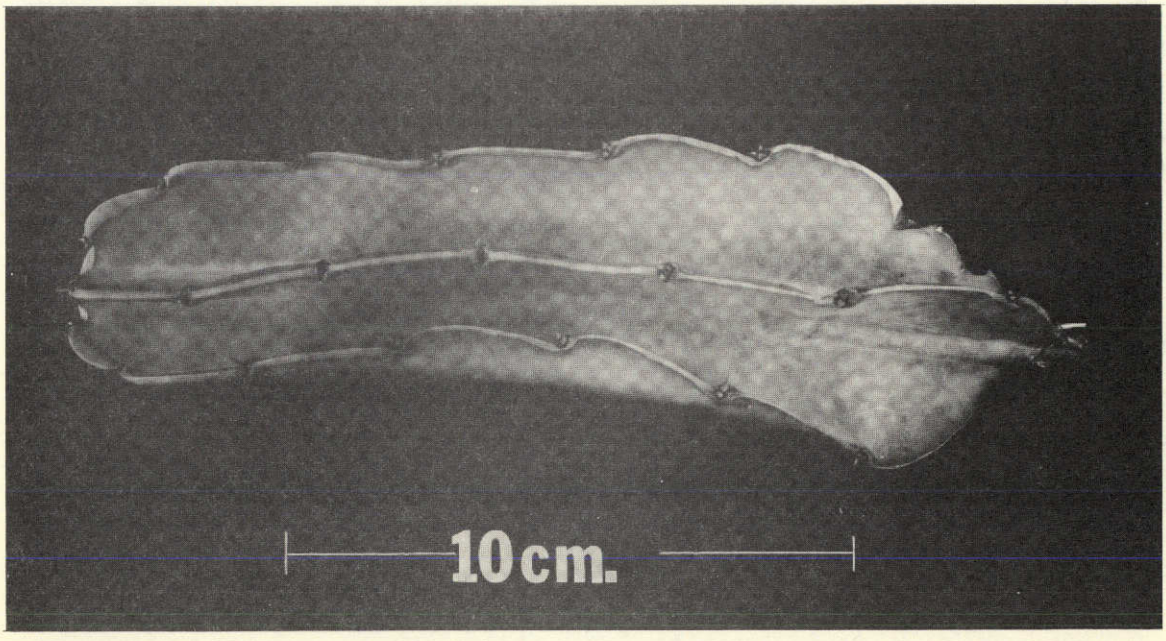

Figure 23. *Hylocereus undatus* (Night blooming Cereus) internode submerged in aerated mineral oil for one month, with no apparent change in outward appearance.